

Figure 1: Isolation of monokaryotic strain deficient in laccase activity.

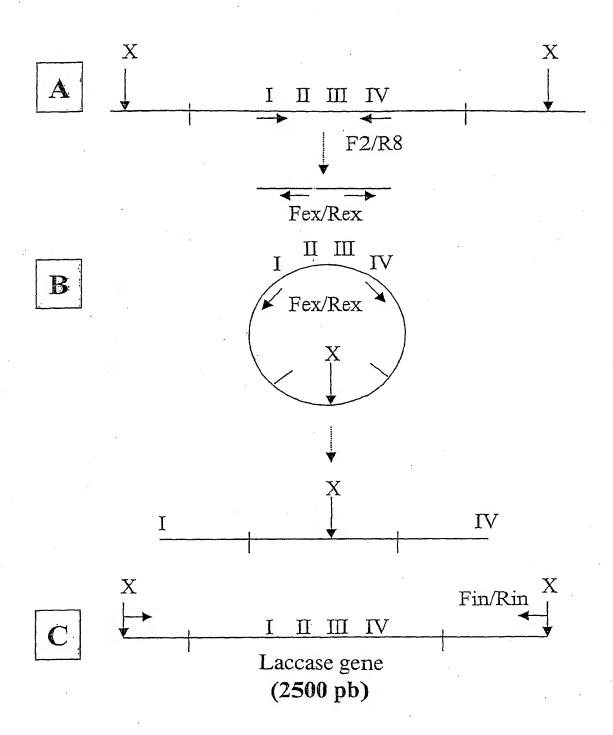
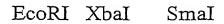


Figure 2: Isolation of the gene coding for the laccase of *Pycnoporus cinnabarinus* laccase.

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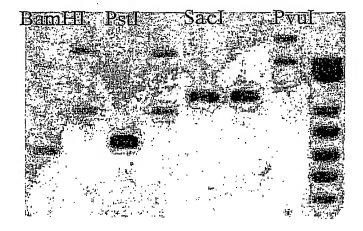


Figure 3: Southern blot study of the gene coding for the laccase of *Pycnoporus cinnabarinus*.

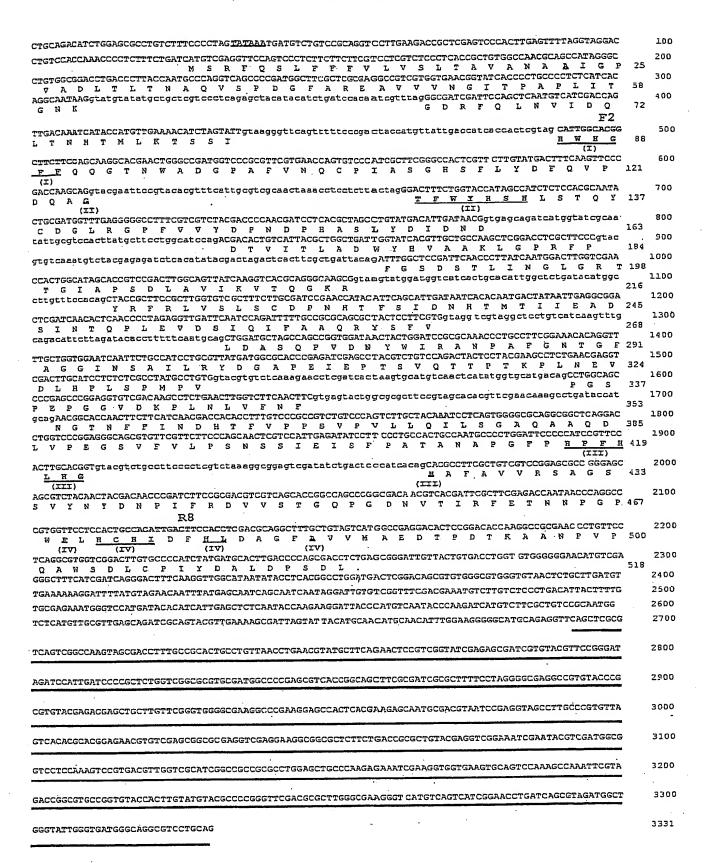


Figure 4: Sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus* 

AGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCAGGGCAGCGACGTA CCGGCCGGCGTGCGCCATTGAGGTACATGAGCGGGGCGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCG CGGCGATGAAACGTTTCCCACCATTGGGAAGAAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAAGGC GGCGTCGCGCTTTGCCGCAGAGGCCGGCGGCGACATGCACAGCGAAGGTCCGTTGCGGATGGGAAGCAGG CAATCAGTGGGTGTCCTACGCCGCCACGATGGTCGGGGAGCGTAGGCGCCCTCCCATAAGGCGGCAAGCATC ATGATGCTCTCCGATTCGGGAAGCCTGGTGCGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACC ATCGCCATCCTCAGCCTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCCTAGATGTGAGCGGGC TTCTTGGATGATCATGTCGTAACTTTTTCTGACCTCGTCGGTGGTACGCATGGCAGGATTGAGCATTACGGT ATGCCTCCCATTCATAAACGATAACCCCTTCCTTCAGGTTGGTCATCTCCATAGAGCGGCACGCTCTCAAGG CCTAGGCTATTCACACCTCCTTCGCAACATCCCTATTCACGGTGTCTGTAAGGAACGACTTGTCATGGGATC ACATGAAGTGCAGCATACTGTTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGT TCAGTCACCACATGGCAAAAAAGCTGCACCATACTCTTTATGGTGAGTTGTTCGTGAGTGGTATACAGTCAT TCATGAGGGAATGCCCACCGGATAGGGTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGT CCTTGTTCAATGAATATCATGGGTCACATGTGGAGACGGTTAAACAGCGTTGACTGTGAATCCCTGGTGTGT GTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCCAGTTCTTTGCGAGCGGCACAG GCAGGCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGG AGCGAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCCTCTCTCACCATTGGGAAGAT TGGACAAGGCCGAGCTATGATAGCTTGCTCCCGAAGTTGGTAAGTCCCGCAATCTGCGGTTCAGGCAACAGT CTCGGAAAAATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAATGCGCACACACGGAGGCTTTA CATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGTCT CTCATGACGTCCGCAATCCAGACCCTTAGCCGGTTCGTTACTCATCGTTATCCCTGCCGCCATGGTAGTGGA GTCAGCCTGGCCAGTGCGTAGTCCCGTCTCTCTTGCTGCACTAGAGAGACCCCCATGAGACAGCGTTTTTTGC TTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTGCACGCCCAGAGGTATTGGGCTCGTCA GATTCCCAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAAATGCTATAGCCCTT CTTCGCGCGACAGCCGCCTTTCAGGGCAAGATAGATCCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTAC CGAACAATTGACTTACCGACATCCTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCCC GCCTCTTTTCGCTCTCACGCATTCCGTGTGGTTCGCGCGACGGCCGCTCATCAGGACCAGACCAGTCTCAAT GTCTGGTACCGGCACAATGGTGACACTGCGGĈAACTGAGTAGGTCTGGTCACTCTGGTGCACCGTCGCTTAC GATCATG

Figure 5: Sequence of the promoter sequence of the gene coding for the laccase of *Pycnoporus cinnabarinus* (up to the ATG coding for the methionine of the laccase).

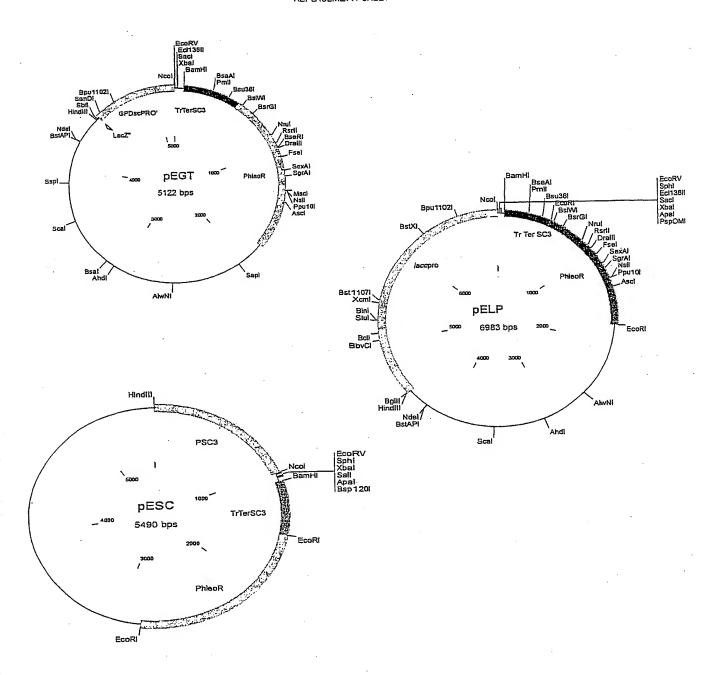


Figure 6: Restriction map of the three expression vectors used for the production of laccase in *Pycnoporus cinnabarinus*.

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CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCGCCTTAAGACGCGTGGATCCGCAGGTGAAC GCGCCTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGT CATAGGGTCGCGGACAAGTGATCGTCTTGCTACATACTCCAAGGTGTTGACTCATTCCCTCGATAATGAACATTGTTGTTGTTTG TTCTCTATCCGCTCAGTCACGCGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACACCGCATGACGACATGGCGAACCTAAG GGGGGTACAAAAGGAGGGTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCT CAAAAAGGTTCTGCCCGTACGCCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATC ATTTTGGGATCGTACAATTATTAGACATGTTGTACAACGTTACATTCCTTTCTTCTTTTACTCTCCGGCCCAGTCTATGTAGAGGTAAA GTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCCGTCTTGCTTCGCCGCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACG AGCAGGTCGCAGACACGGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTTCACGAAGCTCTTGCTGATCGCGATGCCG GGGATCGATCCACGCGTCTTAAGGCGGCCGCGGTACCCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGGTGTTGGTCGGCGTCGG CTCGGTCATGGCCGGCCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCAC CCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGGACCACCCGGC CCGGAACGGCACTGGTCAACTTGGCCATGCATGGTGATGGGCATTATGTGTGATGGGATGCGATGGGAGAGGGAAGTGCTCTGGATG CCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGAAGAACAGTCCTTCGCAGTCCTTCTCGCACC TGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTAATCTCACACCGAAGAGGCTCTCGCGC CACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCCGGGAATTCGT AATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACATACGAGCCGGAAGCATAAAGTGTAAAG CCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCT GCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCG GTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAA CATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACG AGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCC CTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTC ACGCTGTAGGTATCTCAGTTCGGTGGAGCTCGCTCCAAGCTGGGCTGTGCACGAACCCCCGTTCAGCCCGACCGCTGCGCC TTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGA GCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCT GCAAGCAGCAGATTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAA TCTAAAGTATATGAGTAAACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTC ATCCATAGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGGGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCG  $\tt CGCCTCCATCCAGTCTATTAATTGTTGCCGGGAAGCTAGAGTAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCT$ AGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAG TGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACGGGATAATACCGCGCCCACATAGCAGAACTTTAAAAGTGCTCATCATT GGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGA TCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGAC ACGGAAATGTTGAATACTCATACTCTTCCTTTTTCAATATTATTGAAGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTG AATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCA TGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGCTTTCGGTGATGACGGTGAAAACCTCTGACACATGC AGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGG TGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTGTGAAATACCGCACÁGATGCGTA AGGAGAAAATACCGCATCGGCCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGTGCGGGCCTCTTCGCTATTA CGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGAC GCGGGCCCCGCGCCCCCCTCGGCCAGCGGGTGTATCTACGAACGGAACTGGGAGGCGACTCGGAAGAGTTTGGTTAGAAAGGG GAACACCATCGCGGACGGCCCAGTGCTCTGGDCAGCTGAGCGTGCATTGTGTTCAATTCTGACCTGTGGCATGTAAGGAACGTGCTC GGGATCGGAGGGTGGCGCGAGAGCCTCTTCGGTGTGAGATTAGTAACTGTACTGCGAAGCCGCGGAGGGGTTAGGATGAGAGGTAG ACAGGGTCGCAGCCCAGGTGCGAGAAGGACTGCGAAGGACTGTTCTTCGACCGCGCACCTGCAATTGCGCGCATGGATAGAATAGA CTTTCTCCAGCACTCCCATCCAGAGCACTTCCCTCTCCCATCGCATCCCATCACACAATAATGCCCATCAC

**Figure 7:** Nucleotide sequence of the vector pEGT, containing the gpd gene promoter (4480-5112), a phleomycin resistance marker (507-1822) and the sc3 gene terminater (71-507).

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AGCTTCTCCGGCCCGAATCGAACGGCAGGATGTGTGGGCGTGTCCAATATTGCCATGAAAATCTGTCAGAAGTGAGCCCTCTCGTCAC CCTGTACAGCTTCGCTGAGTTGAAAAGCAGGGTTCATCTTGGGCTCACTGATGCACTGAGCTCGACCGGAGAACTAAATGACCAGCCGG AGTGTTCACTAACTTAACGCCGGGTATTCAGGGCAGCTTCTCTATGTTGCGCCTACGACGTAGATCACCGCCCATGAACGGGGGAAACG GGGAGGGGTGCGTTTGGTACGTCTTTACGTCTGGCTATGTTGTATTGACCAGCGTCTGCAGAAGATGGGCACGACGATGCGCCGAGCCG GCCAGTGTCGTCGGATGTCCACTGTTGAGGCCATCCTTTTGCTAGA CAGACGGAAGAGCTTTGGAGGTGCGATTCCTCTACGAATGGGA AGGGGCTTAGATGGAGAGTGACACGTCTGAGCTCCCCAACACGCCTTCGCCGAGGGTGCGTCTCCGCGGACATTCACCTCAGTTCATTG TTCTGACCTGCCTAATTGTATAGACCGGCCAACAACCTTGCTGACGCCCATCATAACAGTGCCCTGCACAGAGCCTTCCCACTCAGTCGG CGCCTCCCTCAATCAATCCCACTAACTCGCCGGCTCTGCCCCTTCGCCGCTCGACACGTCGCTTGGAAGAGCCCGGGCACGGGCGTCCGC AACGCGCGGAAGAAATAATTTACGGGAGCCTCCCCAGGTATAA.AAGCCCCTCACCCGCTCACTCTTTCTCCAGTCGAACACCCCAGT TCAACTACCCAGCCCTTCCTTCCCTATCCTTCYTTACAACCTGCTCGCCATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGAC GGGCCCGGTACCGCGGCCGCCTTAAGACGCGTGGATCCGCAGGTGAACGCGCCTATCGGTGGGÄTATTCGGGCGACGGGAGCCTCGGC AATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGTCATAGGGTCGCGGACAAGTGATCGTCTTGCTACATACTCCAAG TTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAGTAAA GGCTGAGTCGTGGACTAAAGCACTCCACTTTACGGCGAGGATGC CAGTCTACGTCATGAATGAAGCCTCAGGTCCCGAAGTAAGGGGGTACAAAAGGAGGGGTGAAAGGTGGACGTTTTCTTACCATCCTTCCA CCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCTCAAAAAGGTTCTGCCCGTACGCCCGCGAAATTCCTTCGAGGTGGCCCCTATCG CATACATGCACGACTTCAAAACATCCATTCTATCATTTTGGGATCGTACAATTATTAGACATGTTGTACAACGTTACATTCCTTTCTT TTACTCTCCGGCCCAGTCTATGTAGAGGTAAAGTACAAGCGTCCAAAGGATCAGGCACTTAGAGCGCGCCGTCTTGCTTCGCCGCTTAG AGCGCGCCGTCCTGCTTCGCCGCGTAGACGAGCAGGTCGCAGACACGGCGGGAGTAGCCCCACTCGTTGTCGTACCAGGCAATGAGCTT CACGAAGCTCTTGCTGATCGCGATGCCGGGGATCGATCCACGCGTCTTAAGGCGGCCGCGGTACCCCCTCGGACCCGTCGGGCCGCGTC ACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGGACCGCGCTGATGAACAGGGTCACG GCCCTGGTCGAGTCCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGAAGAACAGTCCTTCGCAGT CCTTCTCGCACCTGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTAATCTCACACCGAAGAG GCTCTCGCGCCACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTCARATCAGATCCCCGG TAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCA GCTGCATTAATGAATCGGCCAACGCGCGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTC GGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAAC ATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGC ATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTG CGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTA GGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTA ACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTA GGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTT CGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGATTTT TTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCGTC GTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTT GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTTG GTATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTC CTCCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGT AAGATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAAT ACGGGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTAC CGCTGTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAA AAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTTCCTTTTTCAATATTATTGA AGCATTTATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTAGAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCC CGAAAAGTGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGC GCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGAC CATATGCGGTGTGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGA AGGGCGATCGGTGCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGT TTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGCCA

Figure 8: Nucleotide sequence of the vector pESC, containing the sc3 gene promoter (1-1033), a phleomycin resistance marker (1540-2855) and the sc3 gene terminater (1104-1540).

CATGGGATATCGCATGCCTGCAGAGCTCTAGAGTCGACGGGCCCGGTACCGCGGCCCCTTAAGACGCGTGGATCCGCAGGTGAACGCGC CTATCGGTGGGATATTCGGGCGACGGGAGCCTCGGCAATCTGAGCCTCGTTACTGCCTAGCAAATTCGGAATCCCTTCGATGTCATAGGGT TCAGTCACGCGACCCCACACGTGCATGGTTGAACTTCGCCACGCAACAACCGCATGACGACATGGCGAACCTAAGTAAAGGCTGAGTCGT GTGAAAGGTGGACGTTTTCTTACCATCCTTCCACCTCCCAGACCACCATGCCGGGAATTCCCAGCTTGCTCAAAAAGGTTCTGCCCGTACG CCCGCGAAATTCCTTCGAGGTGGCCCCTATCGCATACATGCACGACTTCAAAACATCCATTCTATCATTTTGGGATCGTACAATTATTAGA CATGTTGTACAACGTTACATTCCTTTCTTCTTTTACTCTCCGGCCCAGTCTATGTAGAGGTAAAGTACAAGCGTCCAAAGGATCAGGCACTT AGAGCGCCGCCTTTGCTTCGCCGCTTAGAGCGCGCCGTCCTGCTTCGCCGCGTAGACGAGCAGGTCGCAGACACGGCGGGAGTAGCCCC ACCCCCTCGGACCCGTCGGGCCGCGTCGGACCGGCGGTGTTGGTCGGCGTCAGTCCTGCTCCTCCGGCCACGAAGTGCACGCAGTTG GACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTTGTCCGGCACCACCTGGTCCTGG TGATGGGATGCGATGGGAGAGGGAAGTGCTCTGGATGGGAGTGCTGGAGAAAGAGGGGAGACGGCGGGGGGGCGCCCTTTTATACCCACG CCGTCGGGCGCCACCACCAGCCCTGGTCGAGTCCCCCTCGAGGGCGACGCTCTATTCTATCCATGCGCGCAATTGCAGGTGCGCGGTCGA AGAACAGTCCTTCGCAGTCCTTCTCGCACCTGGGCTGCGACCCTGTCTACCTCTCATCCTAACCCCTCCGCGGCTTCGCAGTACAGTTACTA ATCTCACACCGAAGAGGCTCTCGCGCCACCCTCCGATCCCGAGCACGTTCCTTACATGCCACAGCGTCAGAATTGAACACAATGCACGTC ARATCAGATCCCCGGGAATTCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCC AACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTATTGGGCGCTCTTCCGCTTCCTCGCTCACTG CAGGAAAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCC CCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAG CTCCCTCGTGCGCTCTCCTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGCTTTCTCATAGCT CACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTT ATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAG GTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGGACAGTATTTGGTATCTGCGCTCTGCTGAAG TTACGCGCAGAAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAACGAAAACTCACGTTAAGGGA TTTTGGTCATGAGATTATCAAAAAGGATCTTCACCTAGATCCTTTTA.AATTAAAAATGAAGTTTTAAATCAATCTAAAGTATATATGAGTA AACTTGGTCTGACAGTTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTCGTTCATCCATAGTTGCCTGACTCCCCG TCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCCCAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATT GGGAAGCTAGAGTAAGTAGTTCGCCAGTTAATAGTTTGCGCAACGTTGTTGCCATTGCTACAGGCATCGTGGTGTCACGCTCGTCGTTTGG TATGGCTTCATTCAGCTCCGGTTCCCAACGATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAAGCGGTTAGCTCCTTCGGTCCT CCGATCGTTGTCAGAAGTAAGTTGGCCGCAGTGTTATCACTCATGGTTATGGCAGCACTGCATAATTCTCTTACTGTCATGCCATCCGTAA GATGCTTTTCTGTGACTGGTGAGTACTCAACCAAGTCATTCTGAGAATAGTGTATGCGGCGACCGAGTTGCTCTTGCCCGGCGTCAATACG GGATAATACCGCGCCACATAGCAGAACTTTAAAAGTGCTCATCATTGGAAAACGTTCTTCGGGGCGAAAACTCTCAAGGATCTTACCGCT GTTGAGATCCAGTTCGATGTAACCCACTCGTGCACCCAACTGATCTTCAGCATCTTTTACTTTCACCAGCGTTTCTGGGTGAGCAAAAACA GGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACACGGAAATGTTGAATACTCATACTCTTTCCTTTTTCAATATTATTGAAGCATT TATCAGGGTTATTGTCTCATGAGCGGATACATATTTGAATGTATTTA.GAAAAATAAACAAATAGGGGTTCCGCGCACATTTCCCCGAAAAG TGCCACCTGACGTCTAAGAAACCATTATTATCATGACATTAACCTATAAAAATAGGCGTATCACGAGGCCCTTTCGTCTCGCGCGTȚTCGG TGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCA GGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCTGGCTTAACTATGCGGCATCAGAGCAGATTGTACTGAGAGTGCACCATATGCGGTG TGAAATACCGCACAGATGCGTAAGGAGAAAATACCGCATCAGGCGCCATTCGCCATTCAGGCTGCGCAACTGTTGGGAAGGGCGATCGGT GCGGGCCTCTTCGCTATTACGCCAGCTGGCGAAAGGGGGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACG ACGTTGTAAAACGACGGCCAGTGCCAAGCTTAGATCTCCGAACCAGAAATGCGATTGCGTTCAGGCCCAATTAAGAATAAAGCTGCGTCA CGGCGTGCGCCATTGAGGTACATGAGCGGGGGGAAAGTCCGCCATTGGTAGCCCTGTCGTGGACGCGGGGGGATGAAACGTTTCCCACCA TTGGGAAGAACGTCTGCGGCCCATCATCCCTTCACCGGATGACAA.GGCGGCGTCGCGCCTTTGCCGCAGAGGCCGGCGGCGACATGCA

Figure 9: Nucleotide sequence of the vector pELP, containing the laccase gene (promoter 4457-6983), a phleomycin resistance marker (507-1822) and the sc3 gene terminater (71-507) (continuation of the sequence on the following page).

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CAGCGAAGGTCCGTTGCGGATGGGAAGCAGCAATCAGTGGGTGTCCTACGCCGCCACGATGGTCGGGGAGCGTAGGCCCCCCCA TAAGGCGGCAAGCATCATGATGCTCTCCGATTCGGGAAGCCTGGTGCGATGCTGGAGAGACTCTCTCCGAGAGACCAGTGTGCGCAAC GTTCCTGGCCTGGAAGACTTTAAAGTGAGTGTAGAAGGGCGAGCAGAGGACGATCATCGGATTGCAGGAACCATCGGCATCCTCAGC CTGGGAAGGATGGCTCTTGGTAGACATTCGCGGAAGGTGTCCTAGATGTGAGCGGGCTTCTTGGATGATCATGTCGTAACTTTTTCTGA CATAGAGCGGCACGCTCTCAAGGCCTAGGCTATTCACACCTCCTTCGCAACATCCCTATTCACGGTGTCTGTAAGGAACGACTTGTCAT GGGATCACATGAAGTGCAGCATACTGTTCGCCGGTCTCGCAGTACAGACGCTAGTACGGGAAGTCGACATCCAAGCGTTCAGTCACCA GTGTGGCGGCCGCAATATTCATCGCCTGGCAATAGTCGATGTGCGTCCTTGTTCAATGAATATCATGGGTCACATGTGGAGACGGTTAA ACAGCGTTGACTGTGAATCCCTGGTGTGTGTTGGGCCGAACAGGTACGTTGCAGGAACACCAATATCTCTTCGGCAGCCCAGTTCTTTG CGAGCGCACAGGCAGGCATCGCGCAACAGATCCCAGCCATCCGGCCTCTGACATTCGGGATACCTGAAGCCCTTCAGGTACGGAGC GAAGAGGTGGGCTCTCTGCAGCGATTGGCGGACGGATAGCTGTATTTCCTCTCTCACCATTGGGAAGATGTGAAAGGCTCCATCATAT GTTGGTAAGTCCCGCAATCTGCGGTTCAGGCAACAGTCTCGGAAA.AATAAGAAGAATATTGTAGGTGCGTGTAGGCGTATCGCCCAAA CCCAGCATCATGTCTCGGCGCAAACTTTACCCTCTATTGACCAACTCCACGAGAAAGCAGGAACAGCTTCCTTGTCTCTCATGACGTCC GCAATCCAGACCCTTAGCCGGTTCGTTACTCATCGTTATCCCTGCCGCCATCGTAGTGGAGTCAGCCTGGCCAGTGCGTAGTCCCGTCT CTCTTGCTGCACTAGAGAAGCCCCATGAGACAGCGTTTTTTGCTTTATTTCTGCTGTTTCTATAGACACCATAGGGGCAAACGATCCTG CACGCCCAGAGGTATTGGGCTCGTCAGATTCCCAGTTTTTCTCCTCGGTCTGAATCGGCTGCACGGCAGATAAATCGGCCGGAAATGCT CGACAGCCGCCTTTCAGGGCAAGATAGATCCTCCCATCATCCCCTACTGCGCTCAGCGCCGGTACCGAACAATTGACTTACCGACATC CTCCGGGACGCGCAAATGCTGTTCGACGGAACGTAATCCTCTTCGTCCCGCCTCTTTTCGCTCTCACGCATTCCGTGGTTCGCGCGA CGGCCGCTCATCAGGACCAGACCAGTCTCAATGTCTGGTACCGGCACAATGGTGACACTGCGGCAACTGAGTAGGTCTGGTCACTCTG 

Figure 9: Nucleotide sequence of the vector pELP (continuation), containing the laccase gene (promoter4457-6983), a phleomycin resistance marker (507-1822) and the sc3 gene ternminater (71-507).

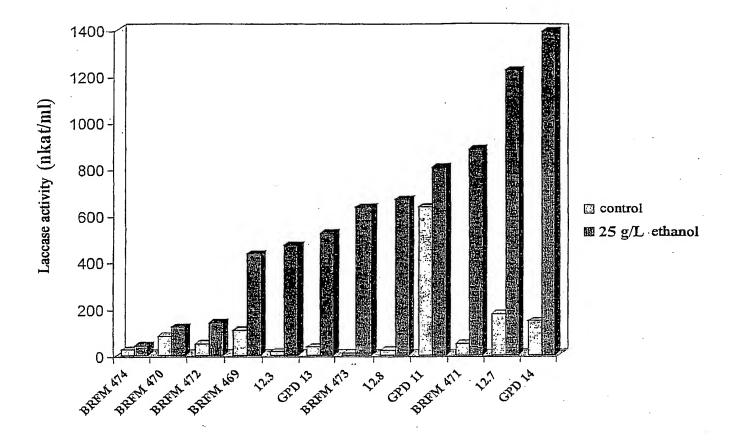
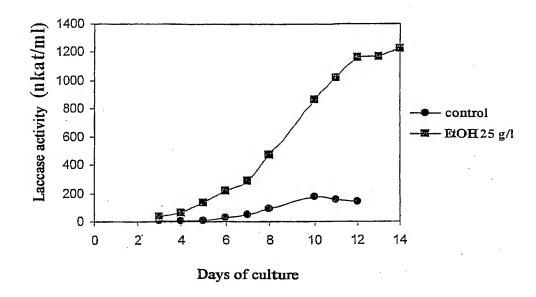


Figure 10: Results of production of the transformants having the most significant activities. The culture was carried out with or without (control) ethanol.



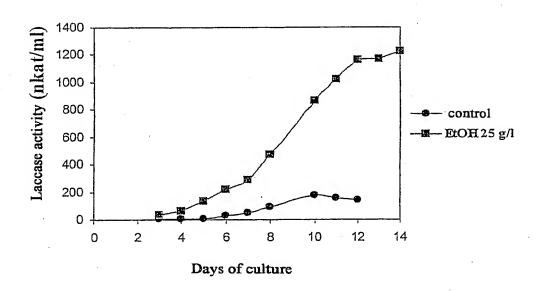


Figure 11: Monitoring of the laccase activities of the transformants GPD 14 and 12.7 as a function of time with or (control) without ethanol

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REPLACEMENT SHEET

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TESCEASCISCCC GCGCC CAAGGAGATA GCATA ATCSCCTGAG AAACCTASTC GTCTCATSGCCGTGT AACCGTICTT GCGACTTATT TTCGCACTTCTC
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                                          MGCLSL
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S V H A A V G P V T D L T L I V D T V A P D G A A F A R E
                                                                       400
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600
800
                                                                        107
900
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                TYDPLDPYRLLYDVDDESTVITLA
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                                                              AGDT
 YHSYAEDILIA
TCCTCATCAATGGTCACGGAAGATTCGCCGGAGCCGGCGGAACGGCAACAGAACTATCTGTCATTACTGTTGAGCATGGAAAGCG<u>GTAGGC</u>ATTCTCCCT 1300
I L I N G H G R F A G A G G T A T E L S V I T V E H G K
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 LRVIEADGITT
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3037
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Gene of the laccase of Halocyphina villosa